

Immunological markers in allergic rhinitis patients treated with date palm immunotherapy

Ghada Boghdadi · Ayman Marei · Atef Ali · Gamal Lotfy ·
Magdi Abdulfattah · Samir Sorour

Received: 9 August 2011 / Revised: 25 January 2012 / Accepted: 8 March 2012
© Springer Basel AG 2012

Abstract

Objective Allergic rhinitis (AR) is regulated by the local production and release of several cytokines. Allergen specific immunotherapy (IT) has been widely used for many years as a specific treatment of allergic diseases. This study aimed to investigate the changes in clinical and immunological markers before and after *Phoenix dactylifera* IT in AR patients.

Materials and methods Total symptom score and levels of total immunoglobulin E (IgE), albumin, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , interleukin (IL)-10, IL-4, and IL-13 were measured in the serum and nasal samples of thirty non-atopic healthy controls and thirty patients with allergic rhinitis before and after 15 months of IT.

Results We found significantly higher concentrations of serum TNF- α and nasal TNF- α and IL-13, and significantly lower concentration of nasal IL-10, in allergic patients than in non-allergic. Moreover, serum and nasal IL-10 increased significantly after IT. However, there was neither a significant reduction in total IgE nor a significant increase in IFN- γ at the end of IT.

Conclusions Our data show a clinical improvement associated with a decline in some inflammation parameters after IT. Moreover, date palm IT induced a significant increase in serum and nasal IL-10 levels.

Keywords Allergic rhinitis ·

Date palm (*Phoenix dactylifera*) · IL-10 · IFN- γ

Introduction

Allergic rhinitis (AR) is a chronic inflammatory disorder of the upper airways that produces characteristic signs and symptoms in sensitized individuals exposed to relevant allergens [1]. Despite advances in immunology, allergic rhinitis remains a significant health issue affecting approximately 30 % of adults and 40 % of children [2]. Furthermore, AR is a symptomatic disorder of the nose induced after exposure to allergens via immunoglobulin E (IgE)-mediated hypersensitivity reactions, which are characterized by four cardinal symptoms of watery rhinorrhea, nasal obstruction, nasal itching, and sneezing [3]. It is associated with a shift in the balance between Th1 and Th2 cells towards a Th2 predominance [4]. The diagnosis is based on clinical manifestations, a positive skin prick test (SPT), elevated serum IgE levels, and/or serum specific IgE antibodies to allergens [5]. Recommended treatments depend on the severity of the disease [1]. Treatments include allergen avoidance, antihistamines, intranasal steroids, antileukotriene receptor antagonists, mast cell stabilizers, and decongestants. However, immunotherapy has been used with success and is the only treatment that may alter disease activity.

Specific allergen immunotherapy (IT) is an effective prophylactic treatment for atopic IgE-mediated disease, in

Responsible Editor: Claudia Kasserra.

Electronic supplementary material The online version of this article (doi:10.1007/s00011-012-0464-4) contains supplementary material, which is available to authorized users.

G. Boghdadi (✉) · A. Marei
Immunology Research Lab, Faculty of Medicine,
Microbiology and Immunology, Zagazig University,
Zagazig, Egypt
e-mail: gsboghdadi@zu.edu.eg

A. Ali · G. Lotfy · M. Abdulfattah · S. Sorour
Faculty of Medicine, Otorhinolaryngology,
Zagazig University, Zagazig, Egypt

particular for severe seasonal allergic rhinitis. A variety of immunological changes have been reported following IT [6]. These include an increase in the allergen-specific IgG antibody [7], a reduction in the allergen-specific IgE antibody subsequent to transient increase [8], and a decrease in quantity and activity of basophils and mast cells [9]. Enhanced interferon (IFN)- γ responses to allergen have been observed after grass pollen IT. Moreover, parallel decreases in skin and nasal late-phase responses to allergen challenge suggest that IT may induce in mucosal T cells a preferential deviation of Th2 in favor of Th1 responses [10].

Grass pollen is a major cause of allergy throughout the world. A number of controlled trials have demonstrated the efficacy of IT in subjects allergic to grass pollen, most using extracts of multiple grasses but some using extracts of a single grass. The optimal grass extract for IT has not yet been established [11]. Date palm (*Phoenix dactylifera*) is commonly planted in the Middle East, neighboring countries (Mediterranean, central Africa, western Asia), Australia, and North America for fruit. Their grains are potent allergens [12, 13]. The common offending allergens causing allergic rhinitis in Egypt are house dust mites and tree pollen. A retrospective study of 1,000 patients undergoing SPT performed at the Allergy and Immunology Unit of the Zagazig University, Egypt, between January 2009 and February 2010 revealed that the local population is more sensitized to outdoor than indoor allergens, especially *Phoenix dactylifera* allergen (unpublished data).

The clinical efficacy of immunotherapy has been demonstrated in patients with pollinosis but little is known about the effect of *Phoenix dactylifera* IT in patients with allergic rhinitis. For these reasons, we aimed to study and characterize the nasal inflammation in date palm allergic rhinitis patients and to investigate the clinical and immunological changes after 15 months of *Phoenix dactylifera* immunotherapy.

Methods

Subjects

This study was carried out at the Departments of Otorhinolaryngology and Microbiology and Immunology of the Faculty of Medicine, Zagazig, Egypt between August 2009 and December 2010. The study started in the pollen season and finished at the end of the second consecutive pollen season. 30 non-atopic healthy volunteers (ages 18–42 years, 14 females and 16 males) and 30 patients (ages 18–36 years, 17 females and 13 males) with allergic rhinitis to date palm pollen were studied. The study was approved by the ethical committee of the hospital and

written informed consent was obtained from each individual. A detailed clinical history and a complete physical examination were carried out for each patient. Each subject in the non-atopic group was chosen on the basis of the following criteria: no history of allergic disease or nasal diseases, no pregnancy or lactation, and negative skin prick test. Each subject in the allergic group was chosen randomly from allergic rhinitis patients attending the allergy outpatient clinic on the basis of the following criteria: history of persistent rhinitis for at least 2 years, positive skin prick test to *Phoenix dactylifera* pollen only (>5 -mm wheal) and no evidence of treatment with IT during the previous 10 years.

Symptom score

The allergic patients recorded the nasal symptoms of obstruction, rhinorrhea, sneezing, and itching 1 week before IT and at the end of the IT course (15 months later). The following scale was used to score each symptom: 0 = no symptom, 1 = mild (symptom was present but was of short duration and not annoying or troublesome, 2 = moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep), or 3 = severe (symptom was troublesome and interfered with normal daily activity or sleep). The total nasal symptom score (TNSS) was the sum of the scores for the individual symptoms. TNSS values (0–12) were categorized as mild (0–4), moderate (5–8), or severe (9–12) [14].

SPT

SPTs were performed at the volar site of each forearm by the same experienced personnel by the application of one drop of each allergen extract of a panel containing house dust mite (*Dermatophagoides pteronyssinus*), *Aspergillus* species mix, cottonwood mix, ash mix, tobacco leaf, ryegrass, and *Phoenix dactylifera* pollen (AL, Allergy Laboratories, Inc., USA) to the skin, at least 3 cm apart, according to a previously validated protocol [15]. Histamine was used as positive control and diluents of each allergen as a negative control (AL, Allergy Laboratories). The sensitivity of the skin test was estimated by the size of the wheal. The largest diameter of the wheal was evaluated as the size of the wheal after 20 min. A wheal diameter 3 mm or greater, accompanied by erythema, was defined as a positive reaction.

Nasal samples

Nasal mucosal samples were obtained by scraping the inferior border of the middle turbinate by a nasal curette-probe and the samples were placed in Eppendorf tubes with

phosphate-buffered saline (PBS). The samples were then homogenized by ultrasonication for 2 min and stored at -70°C until the study.

Mode of IT

The treatment followed the manufacturers' instructions. The patients were given subcutaneous injections of standardized *Phoenix dactylifera* pollen extracts. Injections were administered twice weekly up to a maintenance dose, and thereafter injections were administered twice monthly. The injection period of our study lasted 15 months. The maximum tolerated dose of 1:1,000 of 5 % *Phoenix dactylifera* pollen extracts preparation was attained in 9 months. After each injection, the patient was asked to remain under our supervision for a minimum of 30 min and to report any symptoms they may have experienced. Only oral antihistamines and mid-potency topical steroids were used concomitantly with the IT during the pollen season. However, medication was withdrawn at least 4 weeks before drawing blood and nasal samples.

Determination of total IgE, albumin, IFN- γ , TNF- α , IL-4, IL-10, and IL-13

Blood samples taken from the patients, before and after IT, and healthy controls were kept at room temperature for 30 min till they coagulated. They were then centrifuged at 2,000 rpm for 10 min and serums were obtained. The serum samples were kept at -70°C until the study. Total IgE, albumin, IFN- γ , tumor necrosis factor (TNF)- α , interleukin (IL)-4, IL-10, and IL-13 were measured in serum and nasal samples. Total IgE levels were measured by ELISA according to the kit manufacturer's instructions (Ridascreen, A0141, R-Biopharm AG, Germany). Albumin was measured by the dye binding method according to the kit manufacturer's instructions (Pointe Scientific, Belgium). IFN- γ and TNF- α were measured by ELISA according to the kit manufacturer's instructions (Biosource Europe SA, KAC1231, Nivelles, Belgium and RayBiotech, ELH-TNFalpha-001, USA, respectively). The minimal detection levels of cytokines were 0.03 IU/ml for IFN- γ and 30 pg/ml for TNF- α . IL-4, IL-10, and IL-13 were measured by ELISA according to the kit manufacturer's instructions (AviBion Organium, IL04001 and IL10001, Vantaa, Finland, respectively). The minimal detection levels of cytokines were 3 pg/ml for IL-4, and 2 pg/ml for IL-10 and IL-13.

Statistical analysis

For statistical analysis, we used the Mann–Witney *U* test for comparison between different groups and Wilcoxon

signed ranks test for comparison of paired values. A *p* value less than 0.05 was considered statistically significant. Analyses were performed using SPSS software (SPSS Inc, Chicago, IL).

Results

Thirty allergic rhinitis patients to date palm pollen and 30 non-atopic healthy volunteers entered the study as a control. All patients were symptomatic with a mean TNSS of 5.5 ± 1.3 (0–12) at day 0. The most common symptom was rhinorrhea (70 %). Following 15 months of the immunotherapy, there was a highly significant reduction in TNSS to 1.7 ± 2 ($p < 0.001$) (Supplementary Fig. 1). Clinical improvement in symptoms was accompanied by a significant reduction in the size of the immediate cutaneous response.

Levels of total IgE, albumin, IFN- γ , TNF- α , IL-10, IL-4, and IL-13 in serum

The results are shown in Table 1. At baseline, the serum levels of total IgE in AR patients before IT were statistically highly significant compared to those in the control group ($p < 0.001$). No significant differences were observed between total IgE in AR patients before and after IT. Serum levels of TNF- α were found significantly higher in the untreated group than in controls ($p < 0.05$). However, there was no significant reduction in TNF- α levels at the end of the 15 months of IT. Serum IL-10 level was significantly higher in the patients group after IT (400–1,200 pg/ml) than in the patients group before IT (100–1,000 pg/ml) ($p < 0.05$) (Supplementary Fig. 2). Serum levels of IL-4 could not be determined in any of the patients in the study group or healthy individuals. Serum levels of IL-13 were determined in all patients in the study group but could be determined in only 26 of healthy individuals. No significant differences were observed in levels of serum albumin, IL-13, and IFN- γ in the three groups.

Levels of total IgE, albumin, IFN- γ , TNF- α , IL-10, IL-4, and IL-13 in nasal samples

Nasal levels of total IgE, albumin, IFN- γ , TNF- α , IL-10, and IL-13 were measured in non-allergic controls and AR patients before and after 15 months of IT. The results are shown in Table 1. The nasal samples from AR patients before IT contained significantly higher concentrations of total IgE (0–67 IU/ml, $p < 0.001$), TNF- α (0–100 pg/ml, $p < 0.05$), IL-10 (4.5–8.7 pg/ml, $p < 0.05$), and IL-13 (0.4–3.2 pg/ml, $p < 0.05$) compared to the nasal samples

Table 1 Laboratory data for serum and nasal mucosa levels of total IgE, albumin, TNF- α , IL-10, IL-13 and IFN- γ

	Control group	AR patients before IT	AR patients after IT
Serum			
Total IgE, IU/ml	19.5 (6–94)	242.5 (111–369)*	230 (110–360)*
Albumin, g/dl	3.8 (3.7–4.8)	3.9 (3.5–4.9)	3.8 (3.7–4.8)
TNF- α , pg/ml	86 (50–140)	114.5 (0–200)*	91.5 (0–180)
IL-10, pg/ml	510 (120–1,100)	475 (100–1,000)**	670 (400–1,200)*
IL-13, pg/ml	1.5 (1–5)	1.9 (1–5)	2.1 (1–4)
IFN- γ , IU/ml	7.7 (1–20)	5 (3–8)	5 (1–10)
Nasal mucosa			
Total IgE, IU/ml	4.5 (0–10)	36.5 (0–67)*	31 (0–67)*
Albumin, g/dl	0.09 (0.04–0.1)	0.13 (0–0.25)	0.13 (0–0.23)
TNF- α , pg/ml	40 (10–70)	58 (0–100)*	49 (0–60)
IL-10, pg/ml	9.9 (4.3–12.5)	6.2 (4.5–8.7)*	8 (5.6–9.8)*
IL-13, pg/ml	0.5 (0.05–0.9)	0.85 (0.4–3.2)*	0.69 (0.2–3)
IFN- γ , IU/ml	1.2 (0.5–5)	0.9 (0.4–3.5)	1.4 (0.3–4)

Data expressed as median and total range

* Significant differences from control group

** Significant differences from AR patients after IT

from control subjects (0–10 IU/ml, 10–70 pg/ml, 4.3–12.5 pg/ml, and 0.05–0.9 pg/ml, respectively). IL-4 could not be determined in any of the patients in the study group or healthy individuals. Data in Table 1 show that IL-10 levels in AR patients before IT were significantly lower than after IT (4.5–8.7 vs. 5.6–9.8 pg/ml, $p < 0.05$), while TNF- α levels in AR patients before IT were significantly higher than after IT (0–100 vs. 0–60 pg/ml, $p < 0.05$) (Supplementary Fig. 3). In addition, there were no significant differences in IFN- γ concentration between the AR patients before and after IT. However, a slight trend towards increased IFN- γ secretion was found in the AR patients after IT (0.4–3.5 vs. 0.3–4 pg/ml, $p > 0.05$).

Discussion

Atopy is characterized by Th2 cell polarization, including IL-4, IL-5, and IL-13 which play important roles in causing allergic inflammation [16]. Not only the Th2 but also the Th1 response is considered to differ between atopic and non-atopic individuals. IFN- γ secreted by Th1 cells is generally considered to play a reciprocal role against the activities of Th2-type cytokines, because IFN- γ inhibits IgE synthesis by B lymphocytes and the development of Th2 clones [17]. Serum IgE level is often increased in allergic diseases. However, a normal serum IgE level does not mean that atopy does not exist. On the other hand, a very high serum IgE level can be considered as one of the cardinal markers of allergy in patients without parasitic infection [18]. Studies of AR due to *Phoenix dactylifera* are few. The effect of *Phoenix dactylifera* IT on peripheral blood T-cell responses remains controversial in Egypt.

The main aim of the study was to test the reproducibility of several inflammatory biomarkers and cytokines in serum

and nasal samples of allergic rhinitis patients to *Phoenix dactylifera*, which is commonly planted in the Middle East and other countries, and to investigate whether immunotherapy could affect the spontaneous synthesis of these markers. We compared the levels of total IgE, albumin, IFN- γ , TNF- α , IL-10, IL-4, and IL-13 in the serum and nasal samples of AR patients with a control group. Moreover, we reported the effects of a course of *Phoenix dactylifera* IT on expression of these markers within the serum and nasal samples of AR patients.

Our data seem to be in agreement with the dichotomy of the predominance of Th2 cells over Th1 cells in allergic diseases [19]. We found higher levels of total IgE in the serum and nasal samples of AR patients than in the control group. The level of IFN- γ in the serum and nasal samples was higher in the control subjects than in patients; however, this result is not statistically significant. Serum and nasal levels of IL-4 were below the determination limit in both the study group and control group.

It is remarkable that IL-10 concentrations in the serum and nasal samples of AR patients were significantly lower than the control subjects. This result is not consistent with other reports indicating an increase of IL-10 in atopic allergy and asthma [20, 21]. However, the roles of IL-10 in allergic inflammation and airway hyper-responsiveness are apparently contradictory. Nevertheless, its evident immunosuppressive properties in animal models and in vitro suggest that IL-10 could function as a therapeutic strategy for treating allergic inflammation and asthma [22]. The involvement of IL-13 in the pathogenesis of asthma has been extensively documented [23]. However, the involvement of IL-13 in allergic rhinitis to *Phoenix dactylifera* is less clear. We found that the nasal levels of IL-13 in AR were significantly higher than those of normal controls. In our study, TNF- α levels of the serum and nasal samples in

AR patients were significantly higher than in the control group. A possible explanation of high TNF- α could be that it is a principal mediator of the acute inflammatory response, including allergic rhinitis [24, 25], and could be related to the lower levels of IL-10. A reduction of IL-10 with the increase of TNF- α could be attributed to the worsening of the allergy. Therefore, from all of these aspects, we demonstrated differences in cytokine profile between AR and non-allergic subjects to *Phoenix dactylifera* in the serum and nasal samples and we underlined the close association regarding the relationship between the immunological markers and allergic rhinitis to *Phoenix dactylifera*, especially at the nasal level.

Grass pollen IT was highly effective in reducing symptoms and medication requirements and there were no significant local or systemic side-effects, which confirm the usefulness of this form of therapy in patients who fail to respond to conventional pharmacotherapy [26]. Studies of AR due to *Phoenix dactylifera* are few. To our knowledge, this is one of the first studies to determine immunological changes in subcutaneous *Phoenix dactylifera* IT in AR patients in Egypt. Following 15 months of *Phoenix dactylifera* IT, there was a highly significant reduction in TNSS, which was accompanied by a reduction in the size of the immediate cutaneous response after intradermal allergen challenge. We found in our study that serum levels of IL-10 were statistically significantly higher in AR patients after 15 months of IT than before IT. On the other hand, we could not determine a statistically significant difference before and after IT in respect of serum levels of IgE, IL-13, TNF- α , and IFN- γ . At nasal level, there was a significant increase in IL-10 and a significant reduction in TNF- α , a proinflammatory cytokine that stimulates growth and differentiation of numerous cells, especially T and B lymphocytes. This finding could be explained by a higher level of inflammatory reaction in allergic patients than in the non-allergic. There was a trend towards IFN- γ , a Th1-type cytokine, increase after IT; however, it was not statistically significant. A possible explanation of this result could be related to the short period of IT with a small sample size (30 patients). Taken together, our results suggest that subcutaneous *Phoenix dactylifera* IT influences the cytokine responses at the nasal level more than the serum level. In conclusion, this study provides evidence that serum and nasal levels of IL-10 can be a sensitive parameter that could correlate with clinical improvement and play a role in follow-up of the disease condition of AR. Further studies on large groups of patients with longer treatment durations should be performed to better confirm these findings and their clinical relevance.

Acknowledgment The authors would like to thank Dr. Rehab Karam for her help with the figures.

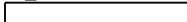
References

1. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol*. 2001;108(Suppl 5):S147–334.
2. Brozek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB, van Wijk RG, Ohta K, Zuberbier T, Schunemann HJ. Allergic rhinitis and its impact on asthma (ARIA) guidelines: 2010 revision. *J Allergy Clin Immunol*. 2010;126(3):466–76.
3. Min YG. The pathophysiology, diagnosis and treatment of allergic rhinitis. *Allergy Asthma Immunol Res*. 2010;2:65–76.
4. Leung DY. Immunologic basis of chronic allergic diseases: clinical messages from the laboratory bench. *Pediatr Res*. 1997;42(5):559–68.
5. Ng ML, Warlow RS, Chrisanthan N, Ellis C, Walls RS. Preliminary criteria for the definition of allergic rhinitis: a systematic evaluation of clinical parameters in a disease cohort (II). *Clin Exp Allergy*. 2000;30(10):1417–22.
6. Passalacqua G, Durham SR. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol*. 2007;119(4):881–91.
7. Rossi RE, Monasterolo G, Coco G, Silvestro L, Operti D. Evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow up of allergic patients undergoing subcutaneous and sublingual immunotherapy. *Vaccine*. 2007;25(5):957–64.
8. Jayasekera NP, Toma TP, Williams A, Rajakulasingam K. Mechanisms of immunotherapy in allergic rhinitis. *Biomed Pharmacother*. 2007;61(1):29–33.
9. Wilson DR, Irani AM, Walker SM, Jacobson MR, Mackay IS, Schwartz LB, Durham SR. Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium. *Clin Exp Allergy*. 2001;31(11):1705–13.
10. Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, Kay AB, Durham SR. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest*. 1993;92(2):644–51.
11. Marcucci F, Sensi L, Di Cara G, Incorvaia C, Puccinelli P, Scurati S, Frati F. Which allergen extract for grass pollen immunotherapy? An in vitro study. *Immunol Invest*. 2010;39(6):635–44.
12. Kwaasi AA, Harfi HA, Parhar RS, Al-Sedairy ST, Collison KS, Panzani RC, Al-Mohanna FA. Allergy to date fruits: characterization of antigens and allergens of fruits of the date palm (*Phoenix dactylifera* L.). *Allergy*. 1999;54(12):1270–7.
13. Postigo I, Guisantes JA, Negro JM, Rodriguez-Pacheco R, David-Garcia D, Martinez J. Identification of 2 new allergens of *Phoenix dactylifera* using an immunoproteomics approach. *J Invest Allergol Clin Immunol*. 2009;19(6):504–7.
14. Schatz M, Meltzer EO, Nathan R, Derebery MJ, Mintz M, Stanford RH, Dalal AA, Silvey MJ, Kosinski M. Psychometric validation of the rhinitis control assessment test: a brief patient-completed instrument for evaluating rhinitis symptom control. *Ann Allergy Asthma Immunol*. 2010;104(2):118–24.
15. Krouse JH, Mabry RL. Skin testing for inhalant allergy 2003: current strategies. *Otolaryngol Head Neck Surg*. 2003;129(4 Suppl):S33–49.
16. Maezawa Y, Nakajima H, Seto Y, Suto A, Kumano K, Kubo S, Karasuyama H, Saito Y, Iwamoto I. IgE-dependent enhancement of Th2 cell-mediated allergic inflammation in the airways. *Clin Exp Immunol*. 2004;135(1):12–8.
17. Maggi E, Parronchi P, Manetti R, Simonelli C, Piccinni MP, Rugiu FS, De Carli M, Ricci M, Romagnani S. Reciprocal

- regulatory effects of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. *J Immunol.* 1992;148(7):2142–7.
18. Worm M, Henz BM. Molecular regulation of human IgE synthesis. *J Mol Med.* 1997;75(6):440–7.
 19. Prescott SL. New concepts of cytokines in asthma: is the Th2/Th1 paradigm out the window? *J Paediatr Child Health.* 2003;39(8): 575–9.
 20. Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q. Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. *Am J Respir Cell Mol Biol.* 1996;14(2):113–7.
 21. Magnan A, van Pee D, Bongrand P, Vervloet D. Alveolar macrophage interleukin (IL)-10 and IL-12 production in atopic asthma. *Allergy.* 1998;53(11):1092–5.
 22. Kosaka S, Tamauchi H, Terashima M, Maruyama H, Habu S, Kitasato H. IL-10 controls Th2-type cytokine production and eosinophil infiltration in a mouse model of allergic airway inflammation. *Immunobiology.* 2010;216:811–20.
 23. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev.* 2004;202:175–90.
 24. Mo JH, Kang EK, Quan SH, Rhee CS, Lee CH, Kim DY. Anti-tumor necrosis factor-alpha treatment reduces allergic responses in an allergic rhinitis mouse model. *Allergy.* 2011;66(2):279–86.
 25. Iwasaki M, Saito K, Takemura M, Sekikawa K, Fujii H, Yamada Y, Wada H, Mizuta K, Seishima M, Ito Y. TNF-alpha contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol.* 2003;112(1):134–40.
 26. Walker SM, Pajno GB, Lima MT, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. *J Allergy Clin Immunol.* 2001;107(1): 87–93.

Figure 1. Reduction in symptom scores after 15 months of immunotherapy. Data expressed as mean $\pm$ SD.

$p < 0.05$



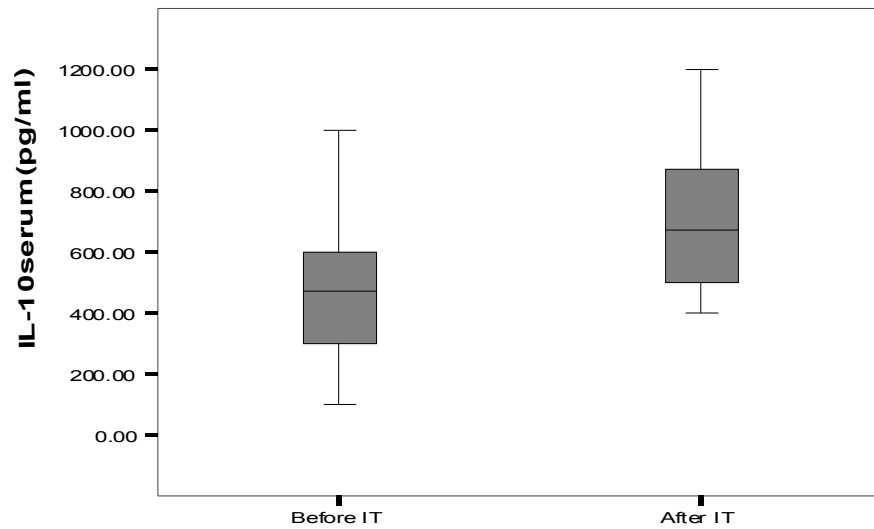
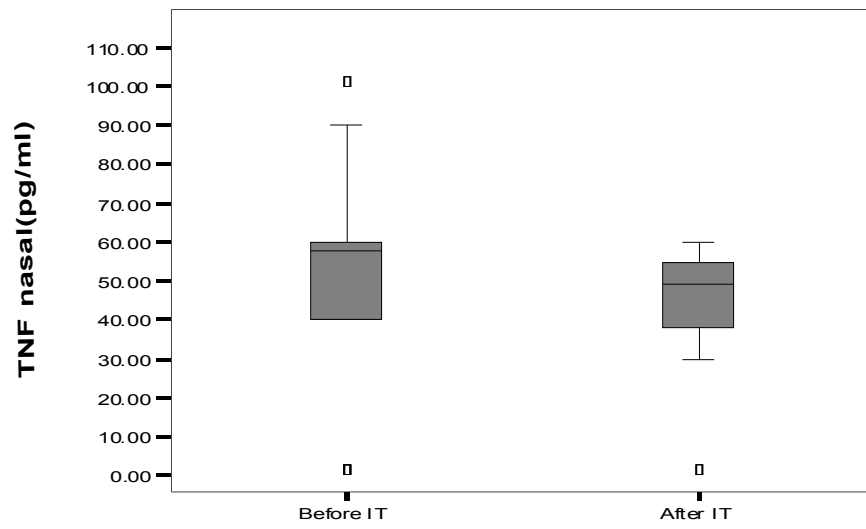


Figure 2. Box and whisker plot of serum level of IL-10 in allergic rhinitis patients before and after IT. Data expressed as median and inter-quartile range.

$p < 0.05$



$p < 0.05$

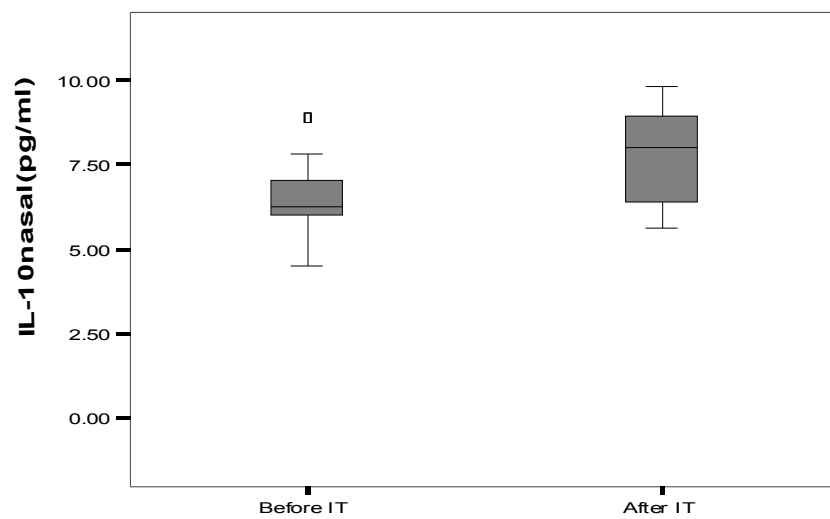


Figure 3. Box and whisker plot of nasal levels of TNF- α and IL-10 in allergic rhinitis patients before and after IT. Data expressed as median and inter-quartile range.

